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CHOLINERGIC REBOUND AFTER CHLORPROMAZINE EXACERBATES LITHIUM MUSCARINIC-INDUCED LIMBIC SEIZURES IN RATS: IMPLICATIONS FOR PSYCHIATRIC TREATMENT[1, 2]

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Summary.—When lithium serum levels were within the (human) therapeutic range, young and old adult male and female rats (housed singly or in groups) all displayed faster limbic seizure onset times in response to a muscarinic cholinergic agonist (pilocarpine 20 mg/kg) if a single systemic dosage of chlorpromazine was injected 24 hours previously. The effect was comparable to injecting an additional 10 mg/kg of pilocarpine. These results strongly suggest that cholinergic rebound from chlorpromazine administrations during lithium treatment could facilitate subclinical electrical lability and very localized neuronal necrosis within the limbic system of clinical patients, resulting in normalization of psychiatric symptoms.

If blood levels of lithium are within the human therapeutic range, rats will display limbic seizures (forepaw clonus) within 1 hr. of the peripheral administration of the muscarinic cholinergic agent, pilocarpine (Honchar, Olney, & Sherman, 1983; Olney, De Gubareff, & Labruyere, 1983; Persinger, Makarec, & Bradley, 1988; Venuogopal & Persinger, 1988). Insidious neuronal loss, associated with electrical and behavioral seizures, then becomes evident over several weeks within the amygdala-hippocampus, several thalamic nuclei and the limbic cortices (Persinger, *et al.*, 1988; Harrigan, Perderey, & Persinger, 1990); one hemisphere is usually affected more than the other.

There is clinical evidence that the severity of the cognitive and affective anomalies in human behavior is: (1) directly related to metabolic activity within the mesiobasal (hippocampal and amygdala) temporal lobes (Andreasen, 1988; Phelps & Mazziotta, 1985) and (2) a function of the proportion of burst-firing limbic neurons that are recruited at any given time (Babb, Wilson, & Isokawa-Akesson, 1987; Isokawa-Akesson, Wilson, & Babb, 1987). This "temporal lobe factor" exists as a continuum along which all human beings are distributed (Persinger & Makarec, 1987). Extreme manifestations enter neuropsychiatric domains.

Traditional treatments of these disorders often involve combinations of pharmacological agents. Except for simple pharmacokinetics and receptor

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binding, the neuromechanisms by which these psychotrophic chemicals affect aggregates of neurons with specific functions is not clear. We are hypothesizing that synergistic effects of some drugs, in this instance, lithium and the neuroleptic agent chlorpromazine, are effective because they induce (cholinergic-mediated) excitotoxin-induced death of the labile populations of limbic neurons that generate the psychiatric behaviors.

During the last five years we have observed repeatedly that cholinergic overshoot from a single injection of strong anticholinergic neuroleptics facilitates limbic seizure onset time (SOT). The effect has been evident within 24 hours after a single subcutaneous injection of between 5 mg/kg and 25 mg/kg of the neuroleptic drug chlorpromazine or CPZ (*Largactil*). Because (1) lithium and chlorpromazine are sometimes used in the polypharmacological treatment of manic disorders, (2) outpatients who receive this treatment may be susceptible to cholinergic rebound because compliance to medication is variable, and (3) we (Persinger & Makarec, unpublished) have found evidence of limbic signs during neuropsychological assessments of patients who are maintained on this drug regime, the present experiment was designed to verify the facilitating effects of CPZ rebound upon SOT for forepaw clonus. It the phenomenon were robust, we reasoned that it should be evident regardless of age and caging conditions.

In Exp. I (the main experiment), a total of 132 Wistar albino rats, 3 to 12 months of age, served as subjects; there were 85 males and 47 females. In Exp. II (dose-dependence study), a total of 48 3-mo.-old male Wistar albino rats were employed. All rats were maintained in standard wire-mesh cages within temperature controlled rooms (LD cycle = 12:12 with onset at 0800 LT). Cage density, which had been established since about 40 days of age, was either 1, 2, or 3 rats per cage. All animals received Ralston Purina Rat Chow and water *ad libitum*.

In Exp. I, the rats were assigned to one of four groups. Each subject received a subcutaneous injection of 3 mequiv/kg of LiCl in the flank 24 hr. before the precipitation of the seizure. All subsequent injections were subcutaneous. Group 1 was injected subcutaneously with 30 mg/kg of pilocarpine while Group 2 received 20 mg/kg of pilocarpine. Group 3 received 25 mg/kg of chlorpromazine (*Largactil*) subcutaneously immediately after the LiCl injection and 24 hr. before injection of 20 mg/kg of pilocarpine. Group 4 received the CPZ 6 hr. before the administration of 20 mg/kg of pilocarpine. The conditions of age and cage density were randomized within the four treatments. SOT, defined as rearing and forepaw clonus, was determined by stopwatch to the nearest second after the injection of the pilocarpine. If the rat did not display the seizure within 70 min., this value was given for its SOT. The rats were tested in groups of 6 to 12 over 13 different days during a 3-mo. period. In Exp. II, rats were injected with either 5 mg/kg, 15 mg/kg

or 25 mg/kg of CPZ or placebo (12 rats/treatment) a few minutes after the lithium injection. Twenty-four hours later all rats received 20 mg/kg pilocarpine.

The means and standard deviations for each sex for each of the four treatments in Exp. I are shown in Fig. 1. Two-way analyses of variance of SOT as a function of the four treatments and sex yielded a statistically significant treatment ($F_{3,119} = 34.95$, $p < .001$) and sex difference ($F_{1,119} = 23.36$, $p < .001$) but no significant interaction. The sex effect was due to the increased SOT of the females relative to the males, an observation that is typical of the phenomenon. *Post hoc* analyses indicated that the primary source of the difference was between the rats that had received the CPZ 24 hr. before the pilocarpine and 6 hr. before the pilocarpine.

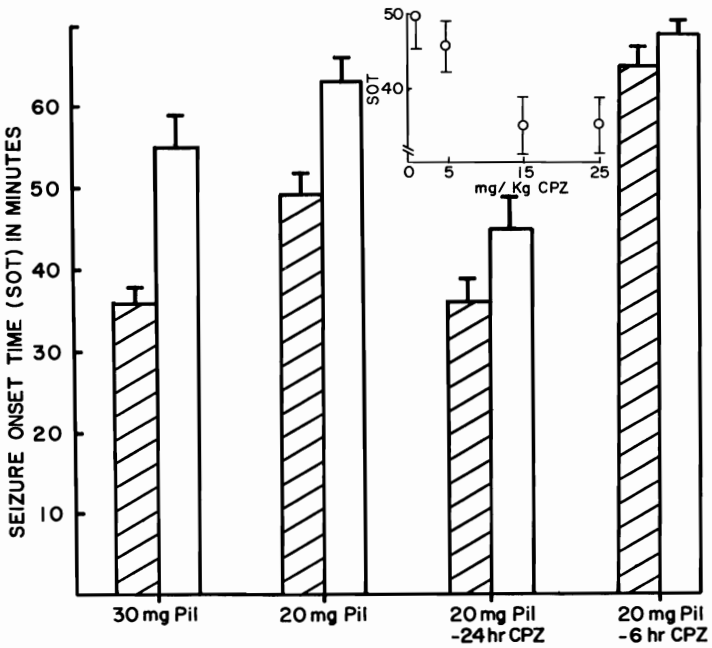

Fig. 1. Mean seizure onset time (SOT), as defined by forepaw clonus and rearing, for male (diagonal bars) and female rats (open bars) that were injected with 3 mequiv/kg of lithium and then: (1) 30 mg/kg of pilocarpine 24 hr. later, (2) 20 mg/kg of pilocarpine 24 hr. later, (3) 25 mg/kg of CPZ and then 20 mg/kg of pilocarpine 24 hr. later, and (4) 25 mg/kg of CPZ 6 hr. before 20 mg/kg of pilocarpine 24 hr. later. Bars indicate standard errors of the mean. Insert shows the results of the study of dose-dependence.

Because the 6-hr. CPZ treatment effectively blocked the SOT, consequent one-way analyses of variance were completed for the other three treatments for each sex (58 males, 34 females). Statistically significant group

differences were noted for both males ($F_{2,55} = 5.54$, $p < .01$; 17% of variance) and females ($F_{3,32} = 6.82$, $p < .01$; 23% of variance). *Post hoc* Tukey's tests ($p < .05$) indicated that the source of the differences was between the group that received the 20 mg/kg of pilocarpine relative to the group that received the 30 mg/kg of pilocarpine and the group that received the CPZ 24 hr. before but only 20 mg/kg of pilocarpine; the latter two groups did not differ significantly from each other for either sex.

There were no significant differences between the treatments in body weights for either males or females; the grand means and standard deviations were 591 (83) gm and 367 (66) gm, respectively. Covariance for age or weight did not change the treatment differences. There were no statistically significant associations (all *r*s between −0.10 and 0.10) between SOT and age or body weight for either males or females. Neither the male nor female rats displayed significantly different SOT between the different test days.

To affirm the robust nature of the effect, analyses of variance were completed for SOT as a function of the CPZ overshoot group ($n = 36$) and the reference group ($n = 38$), cage density and age (dichotomized for 3 to 7 months, 8 to 13 months). As predicted the rats that had received the CPZ 24 hr. before the pilocarpine injection displayed statistically significant faster SOTs ($F_{1,59} = 17.98$, $p < .001$; 20% explained variance); there were no statistically significant main effects or interactions for age or cage density. The mean and *SD* of the SOT for an additional 5 rats, injected with the same dosage of CPZ 48 hr. before the 20 mg/kg of pilocarpine was 61 (12) min. This group was not significantly different from the group that never received any CPZ but only 20 mg/kg of pilocarpine.

The additional dose-dependence results from Exp. II are shown in the insert (Fig. 1). One-way analysis of variance for the four treatments was statistically significant ($F_{3,44} = 3.21$, $p < .01$); the major source of the difference was between the placebo and 5-mg/kg CPZ treatments (that did not differ from each other) and the 15- and 25-mg/kg CPZ groups (that did not differ from each other). The latter two groups displayed quicker SOTs than the placebo or 5-mg/kg CPZ groups; the absolute values of the SOT and the magnitude of the treatment effect were not significantly different from those of Exp. I.

These results clearly suggest that the cholinergic rebound effect from a single injection of chlorpromazine is equivalent to 10 mg/kg of pilocarpine. That both the delay (CPZ + 6 hr.) and earlier onset (CPZ + 24 hr.) of the seizure is associated with cholinergic rather than other transmitter systems is suggested by the lack of comparable effect by equivalent dosages of haloperidol (unpublished observations). Maintained single cage isolation, a procedure known to affect brain stem norepinephrine levels, also did not affect the SOT.

It is important to emphasize that the shorter latency in SOT for rats that had received the 25 mg/kg of CPZ 24 hr. *before* seizure induction occurred despite the low-level lethargy that was displayed by these animals. That this lethargy was an anomalous confounding variable is unlikely because rats that had received 15 mg/kg of CPZ 24 hr. before the seizure did not display residual lethargy but also showed quicker SOTs. The pattern suggests that the protective effect of CPZ against limbic seizures immediately subsequent to injection is associated with a comparable susceptibility to limbic lability a few hours later.

Although the inferred cholinergic overshoot 24 hr. after the injection of 15 to 25 mg/kg of CPZ involved dosages of this neuroleptic that substantially exceed those used for clinical treatment of human patients, the results are considered relevant. It is possible that the optimal rebound time may be less or greater than 24 hr. In addition, there are common correlative pharmacological factors, such as nicotine (cigarettes) or dietary manipulations, that could facilitate cholinergic rebound in patients.

We conclude that our results may have immediate relevance for clinical settings in which lithium and chlorpromazine are given together, especially if the patient has displayed an history of complex partial seizure signs and symptoms. Because they exist as continua, their severity requires quantitative measurements of incidence and actuarial comparisons (Persinger & Makarec, unpublished; Roberts, Varney, Hulbert, Paulsen, Richardson, Springer, Shepherd, Swan, Legrand, Harvey, Struchen, & Hines, 1990), DSM-III—R criteria and clinical impressions would not be discriminative.

If our results are generalizable to human populations, then microseizure activity within the deep mesiobasal temporal lobe structures may occur in patients whose CPZ medication fluctuates upon a therapeutic lithium level. Because conspicuous alterations in cognition frequently require bilateral limbic involvement in human beings and such recruitment is likely to be infrequent, local seizure activity may be evident neither by classic electroencephalographic or clinical criteria; however, subtle long-term changes, evident over several days to weeks, would be expected to emerge.

If the insidious amygdaloid-entorhinal neuronal damage that develops in rats (Persinger, *et al.*, 1988) is comparable to the process that occurs within the human brain, then the efficacy of lithium-neuroleptic combinations in some patients may be related to *selective* necrosis of labile neuronal populations that were facilitating the psychiatric symptoms. This process would certainly not alter the utility and importance of this pharmacological treatment for acute disorders. However, the elucidation of the mechanism might facilitate informed decision, promote quantitative assessment during the years that follow intervention, and help ameliorate the expected negative sequelae.

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